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NaCl-INDUCED CHANGES IN OXYGEN EVOLVING ACTIVITY AND THYLAKOID MEMBRANE PATTERNS OF BARLEY PLANTS. ADAPTATION TO SALINITY

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Summary. The effects of NaCl salinity on photosynthetic oxygen evolving activity and polypeptide membrane profiles were investigated in barley seedlings. High salinity (100 mM NaCl) markedly reduced oxygen evolution in isolated thylakoids from NaCl-treated plants. Lower salt concentrations and stepwise (20–100 mM NaCl) treatment of the seedlings had a lower effect on O_2-producing reactions.

Chloroplasts isolated from prolonged NaCl-treated plants showed enhanced ability to tolerate high (1 M NaCl) concentration after *in vitro* incubation. We consider the observed stabilization in PSII oxygen-evolving activity as a result of adaptation to salinity.

Protein patterns of thylakoid membranes on SDS-PAGE stained with Coomassie revealed that the relative amount of a number of polypeptides were altered when barley plants were grown on NaCl solutions.

The results are discussed as an effect of prolonged NaCl application on the photochemical activity determined by some structural alterations in biomembranes that seems to play a specific role in the adaptive process.

Key words: photosynthetic oxygen evolution, thylakoid membranes, salinity

***Abbreviations**:* ABA – abscisic acid; DCPIP – 2,6-dichlorophenol indophenol; F_{max} – maximal fluorescence; F_v – variable fluorescence; JA – jasmonic acid; PSII – photosystem II; SDS-PAGE – sodium dodecylsulphate polyacrylamide gel electrophoresis

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Introduction

Photosynthesis of many plants decreased drastically as a result of NaCl salinity (Gale et al., 1967; Flowers et al., 1977; Greenway and Munns, 1980). Data relating to the effect of salinity on the primary photochemical reactions under *in vivo* conditions, however, are limited and conflicting (Muller and Santarius, 1978; Robinson et al., 1983; Neale and Melis, 1989; Maslenkova et al., 1991). In the same time many plant species are capable of adapting to extreme salinity. Photosynthetic membranes are stress sensitive sites but little information is available regarding the structural changes associated with salt stress (Pfeifhofer and Belton, 1975; Preston et al., 1987; Carter and Cheesman, 1993) and the exact mechanisms of membrane damage and protection are still unknown. In this respect the analysis of chloroplast structure and function is an important part of the study of NaCl salinity effect on the photosynthetic apparatus. Increase in salt resistance involves a protection of chloroplast membranes by some changes in lipid and protein composition, accumulation of different protector components and enhanced level of phytohormones (Muller and Santarius, 1978; Downton and Loveys, 1981; Walker and Dumbroff, 1981; Maslenkova et al., 1993). It has been shown that exogenous abscisic acid (ABA) facilitates the adaptation to salinity (La Rosa et al., 1985; Amzallag et al., 1990; Maslenkova et al., 1993), but the molecular basis of this effect is still obscure. Judging from the identical polypeptide profiles of thylakoid membranes, isolated from jasmonic acid (JA)-, ABA- and NaCl-treated barley plants and enhanced oxygen evolving activity in ABA-pretreated seedlings before salt application we reach the conclusion that these phytohormones may play a specific role in adaptation to salinity (Maslenkova et al., 1992; 1993).

In this report the effects of NaCl salinity on oxygen-evolving reactions and polypeptide composition of barley thylakoid membranes are examined. It is shown that prolonged NaCl-application to the root medium of barley seedlings increases the stability of membrane structure and function after 1 M NaCl *in vitro* incubation. The results are discussed in term of an effect of salinity on photochemical activity determined by some structural alterations in biomembranes that seems to play a specific role in the adaptive process.

Materials and Methods

Plant material

Barley (*Hordeum vulgare* L., var. Alfa) seeds were germinated for 2 days on two layers of moist filter paper in vermiculite at 25 °C in the dark. Then they were transferred into Petri dishes containing 40 ml distilled water for 2 days. NaCl treatment consisted in transferring the seedlings on equal amounts of water solution from the required NaCl

concentrations for 5 days. The solutions were changed every 24 h. During the experimental period, the seedlings grew in a growth chamber under white fluorescent lamps (35 $W.m^{-2}$), with 12 h light/dark periods. Day/night temperatures were 25/20 °C. Relative humidity was about 50%. To obtain NaCl stepwise treated plants, the daily increase in NaCl concentration was 20 mM until 100 mM NaCl was reached after 5 days.

In vitro treatment of isolated thylakoids was conducted in 1M NaCl solutions for 10 minutes before measurements.

Thylakoid membrane preparation

Barley thylakoids were isolated as described by Camm and Green (1980) and suspended in medium containing 0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 0.2% (w/v) BSA and 50 mM Tricine-NaOH (pH 7.8).

Measurement of oxygen evolution

The activity of electron transport from water to 2,6-dichlorophenol indophenol (DCPIP) was measured at 25 °C in the same medium. The concentration of the artificial electron acceptor was 30 μM and chloroplasts equivalent to 15 μg Chl/ml were used. Reduction of the dye was measured at 580 nm.

Gel electrophoresis

One-dimensional gel electrophoresis was conducted after the procedure of Laemmli (1970) 5% (w/v) acrylamide slab gels (1.5 mm thick), containing 0.1% (w/v) SDS and 375 mM Tris-HCl, pH 8.7. Samples of membrane fractions were solubilized in a sample buffer containing 2,5 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 5% (v/v) β-mercaptoethanol and 10% (v/v) glycerol. Thylakoid membranes were incubated in solubilizing buffer for 30 min at room temperature, SDS/Chl – 20:1. Protein corresponding to 10–15 μg Chl was applied to each lane. After electrophoresis, gels were stained with Coomassie Brilliant Blue R-250. Molecular weights were estimated from a standard plot using lysozyme (14 300), β-lactoglobulin (18 400), trypsinogen (24 000), carbonic anhydrase (30 000), ovalbumin (43 000), bovine serum albumin (66 000) and phosphorylase A (94 000).

Results and Discussion

Table 1 shows inhibition of Hill reaction activity (with DCPIP as electron acceptor) in thylakoids from NaCl-stressed plants with increasing NaCl concentrations. The activity of 100 mM NaCl-stressed plants was considerably lower (68.8%) than the activity of control plants. In 50 mM NaCl and stepwise salt treated plants Hill reac-

Table 1. Iinfluence of different NaCl concentrations on Hill reaction activity* of isolated chloroplasts and chlorophyll *a/b* ratio**

Treatment	Activity (%)	Chl *a/b*
Control	100.0 ± 0.0	2.68 ± 0.18
25 mM NaCl	93.5 ± 3.1	2.60 ± 0.11
50 mM NaCl	79.9 ± 4.7	2.65 ± 0.13
100 mM NaCl	68.8 ± 6.0	2.58 ± 0.11
20–100 mM NaCl	87.9 ± 6.1	2.64 ± 0.13
Control + 1 M NaCl	24.2 ± 4.8	–
100 mM NaCl + 1 M NaCl	49.7 ± 1.7	–
20–100 mM NaCl + 1 M NaCl	33.1 ± 5.9	–

*Oxygen evolution with DCPIP in thylakoids from control (untreated) plant was 51.3 μmol O_2/mgChl.h.

**Values are averages of four separate experiments ± St. dev.

tion activity was less affected (79.9% and 87.9%, respectively). Similar data of inhibitory effect of NaCl salinization were previously reported about electron transport capacity of isolated chloroplasts, fluorescence induction (F_v/F_{max}) and O_2 flash yields (Gambarova et al., 1988; Maslenkova et al., 1991, 1993). Chl*a*/Chl*b* ratio was not changed practically during the treatment, independently of the manner in which salt stress was applied.

Analysis of polypeptide pattern obtained by SDS-PAGE shows that 7-day treatment with 50-, 100- and 20–100 mM NaCl visibly changes polypeptide composition of thylakoid membranes (Fig. 1*a*; see also Fig.1*B* (Maslenkova et al., 1992) and Fig.3 (Maslenkova et al., 1993). Special attention deserve the changes in polypeptides belonging to the photosystem II (PS II) complex. Compared to the control, thylakoids in NaCl-treated membranes show a marked depletion of all PSII polypeptides such as Cpa complex (47 and 43 kDa) polypeptides, 33-, 24- and 16 kDa polypeptides of oxygen evolving complex, polypeptides of LHCII and some other unidentified bands. We also observed an enrichment (evident from the intensity of Coomassie staining) of a polypeptide with an apparent molecular mass 56–57 kDa and some polypeptides in the region of 20.5–15 kDa. The observed alterations could be explained either by the sensitivity of thylakoids towards stress or by the tolerance requiring synthesis of new proteins.

Fig. 1. Influence of 1M NaCl incubation *in vitro* on polypeptide profiles of thylakoids isolated from control plants (1 and 4), 100 mM NaCl treated plants (2 and 5) and 20–100 mM stepwise treated plants (3 and 6). "*a*" – before and "*b*" – after subjection to 1 M NaCl

In order to investigate the existence and extent of adaptation to salinity, isolated thylakoids from untreated, 100 mM NaCl and NaCl stepwise treated barley plants were subjected to high (1 M) NaCl concentration *in vitro* and the oxygen evolving activity and polypeptide patterns were subsequently measured. Table 1 shows considerable inhibition of oxygen evolution after 1 M NaCl incubation of control thylakoids (only 24.2% from the initial oxygen-evolving activity), while the activity in 100 mM NaCl stressed and 20–100 mM stepwise treated plants was less affected (49.7% and 33.1%, respectively). These results and previously reported data (Maslenkova et al., 1993) substantiate the conclusion that prolonged NaCl treatment increased the resistance of chloroplast membranes towards salinity.

Most probably the observed resistance to salinity is in close connection to the changes in membrane structure. The polypeptide profiles in Fig. 1*b* provide evidence that thylakoid membranes from NaCl-treated plants are in fact more resistant to 1 M NaCl treatment *in vitro*. After 1 M NaCl treatment the membranes of control plants lost a great part of their polypeptide bands along with the substantially decreasing Hill reaction activity (Table 1). The ability of chloroplast membranes to evolve oxygen under flash illumination (by Kok's mechanism for O_2 production) completely disappeared, Fig.1 (Maslenkova et al. 1993). Thylakoid membranes from plants post prolonged NaCl treatment and especially in the case of 100 mM NaCl application retain the membrane structure to a great extent after *in vitro* 1 M NaCl incubation parallel with the preserved capacity of isolated membranes to evolve oxygen (Table 1).

As previously shown (Maslenkova et al., 1992, 1993) identical polypeptides were synthesized in thylakoids from ABA, JA and NaCl treated barley plants as well as in thylakoid membranes from ABA pretreated plants before salinization. In addition this pretreatment deminished the inhibitory effect of high salt concentration. It is likely that ABA and salinity trigger one and the same genetic system and that some of these common polypeptides might play a definitive role in adaptation to salinity. Even a minor alteration in the membranes may change the functional connection between reaction centre complexes and antenna complexes. According to our hypothesis (Maslenkova et al., 1989, 1993) these modulations in membrane composition reflect a relative increase in the number of stroma situated PSIIβ centres, functioning by a co-operative oxygen evolving mechanism (Zeinalov, 1982), which is more resistant to the stress. This conversion of the PSII oxygen evolving centres seems to have a physiological significance under environmental stress conditions (Guenther and Melis, 1990), including salinity.

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